for gt

TUES 12-22-92

Attention: Vittorio Sgaramella
Dept. of Genetics and Microbiology
University of Pavia

From: Greg Tombline
Lederberg Lab, Rockefeller University
New York , USA

Dear Vittorio,

You should have probably saved the congratulations... I ran the gel on friday most of the day (approx 7-8 hours) and tried a new but commonly used protocol explained in the Hydro-link booklet, ie., an 8% Hydolink gel containing approx. 30-40% formamide as well as the normal amount of urea and 1x TBE in both the gel and buffer chambers. This protocol supposedly ensures maximum readability and simultaneously should reduce compressions in the "G" lane due to 2' structure. Well, the lanes were perfectly straight, but the bands were very blurry/ fuzzy (after 60 hours exposed- fri -mon morn.). However, although the sequence did reach the proper point for detecting the mutants, the fingerprinting did not show up well at all (even though they were extracted, ppt'd with glycogen, and resuspended in 3ul formamide and loaded completely). So, this is the plan.... I have exposed the gel to the Phosphorimager, in the meanwhile I ordered some alpha 32P dATP to use in the F.P. instead of 35S. We still have some of the sequencing but Ican also make more. I have not been able to do much with the kinased PCR products, but plan to. I will also try using some of the kinased primers in the F.P. Our progress will be somewhat arrested because of the holidays, but I would assume that within a week or so things should develop - I will be leaving tomorrow for Robin's family for Christmas but will return by Sunday. The 32P will be here on Tues, 12/29 - also delayed because of the holiday..

Well for now a' bientot, und haben sie eine shoënus Christmas--- alle tzuzammen.. non prevalebunt. Hello to the girls and Paula.

Ciao,
Greg